510 Rec'd CT/PTO 18 MAY 1999

WO 98/22513

PCT/GB97/03140

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# PRODUCTION OF VEGETABLE GELS

The present invention relates to hemicellulose-based gels and viscous media, to processes for their production, to products containing such gels and/or viscous media and to various applications thereof. In particular, the present invention relates to an improved method for performing oxidative gelation of hemicelluloses which avoids the need for the addition of hydrogen peroxide.

Plant tissue, especially cell wall material, contains hemicelluloses. The term "hemicellulose" is a term of art used to embrace non-cellulosic, non-starch plant polysaccharides. The term therefore embraces inter alia pentosans, pectins and gums.

Some hemicelluloses are suitable as substrates for oxidative gelation ("gelling hemicelluloses"): such hemicelluloses often have substituents with phenolic groups which are cross-linkable with certain oxidizing agents.

Arabinoxylan and pectin constitute two particularly important classes of hemicellulose. Arabinoxylans consist predominantly of the pentoses arabinose and xylose, and are therefore often classified as pentosans. However, in many cases hexoses and hexuronic acid are present as minor constituents, and therefore they may also be referred to descriptively as heteroxylans.

The arabinoxylan molecule consists of a linear backbone of (1-4)-G-xylopyranosyl units, to which substituents are attached through O2 and O3 atoms of the xlosyl residues. The major substituents are single  $\sigma$ -Larabinofuranosyl residues. Single  $\sigma$ -D-glucoronopyranosyl residues and their 4-O-methyl athers are also common substituents.

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Arabinoxylan preparations are usually heterogenous with respect to the ratio of xylose to arabinose (i.e. the degree of substitution) and in the pattern of substitution of the arabinosyl units along the (1-4)-G-xylan backbone.

Phenolic acid (including ferulic acid) and acetyl substituents occur at intervals along the arabinoxylan chains. These substituents to some extent determine the solubility of the arabinoxylan. Arabinoxylan preparations bearing phenolic (e.g. ferulic acid substituents) are referred to herein as "AXF", while those bearing acetyl substituents are designated "AXA". Similarly, preparation bearing both phenolic (e.g. ferulic acid) and acetyl substituents are hereinafter abbreviated to the designation "AXFA". Arabinoxylan preparations having few phenolic (e.g. ferulic acid) substituents are designated "AX": when the degree of substitution falls below that required for oxidative gelation, the arabinoxylan is designated a "non-gelling arabinoxylan" (a term which therefore embraces AX and AXA).

Pectins constitute another important class of hemicelluloses. As used herein and unless otherwise indicated, the term "pectin" is used sensu lato to define hemicellulose polymers rich in D-galacturonic acid. Many (but not all) are cell wall components. The term "pectin" is also used herein sensu stricto to define the so-called "true pectins", which are characterised by the presence of an O-(a-D-galacturonopyranosyl)-(1-2)-L-rhamnopyranosyl linkage within the molecule.

The pectins may be subcategorized on the basis of their structural complexity. At one extreme are "simple pectins", which are galacturonans. At the other extreme are "complex pectins" exemplified by rhamnogalacturonan II, which contains at least 10 different monosaccharide components in the main chain or as a components of branches. Pectins of intermediate complexity (herein referred to as "mesocomplex pectins" contain alternate rhamnose and galacturonic acid units, while others have

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branches of glucoronic acid linked to galacturonic acid.

Complex and mesocomplex pectins are made up of "smooth" regions (based on linear homogalacturonan) and "hairy" regions corresponding to the rhamnogalacturonan backbone with side-branches of varying length.

Certain pectins (for example, pectins obtainable from representatives of the plant family Chenopodiaceae, which include beets (e.g. sugar beet), spinach and mangelwurzels) are substituted to some extent with substituents derived from carboxylic acids (usually substituted cinnamic acids) containing phenolic groups. Such pectins may be oxidatively crosslinked to produce viscous solutions or gels via their phenolic substituents. This can be achieved by powerful oxidants (e.g. persulfate - see J. - F. Thibault et alia, in The Chemistry and Technology of Pectin, Academic Press 1991, Chapter 7, pages 119-133) or a combination of peroxidase and hydrogen peroxide (see Thibault et alia, ibidem). FR 2 545 101 A1 also describes the gelling of beet pectins using an oxidant (e.g. hydrogen peroxide) and an enzyme (peroxidase). Such pectins are referred to herein as "gelling pectins".

Sugar beet pectin is especially rich in arabinan. Arabinan contains G-1, 5-linked arabinose in the backbone with g-(1->3) or g-(1->2) - linked arabinose residues, whereas arabinogalactan contains G-1, 4-linked galactose in the backbone, with g-(1->3) or g-(1->2) linked arabinose residues. Ferulyl substituents are linked to the arabinose and/or the galactose in the arabinan and arabinogalactan side-branches of the rhamnogalacturonan part. The "ferulic acid" content varies according to the extraction method, but is often about 0.6%.

Beet pectins obtained by processes which partially remove arabinose residues may exhibit improved gelling properties. Thus, procedures involving mild acid treatment and/or treatment with an a-arabinofuranosidase will

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improve the gelling properties of the pectin (see F. Guillon and J. -F. Thibault, ibidem). Such pectins are hereinafter referred to as "treated pactins". Hemicelluloses are complex mixtures of noncellulosic cell wall polysaccharides, including pentosans such as arabinoxylans. Convenient sources of hemicelluloses include cereals (such as maize, barley, wheat, oats, rice), pulses (e.g. soya), legumes and fruit.

There are many known methods for fractionating plant material (such as testaceous or cell wall material) to produce hemicellulose and cellulose fractions. Such methods usually involve alkali or water extraction to yield insoluble cellulose and soluble hemicellulose fractions, followed by separation. The soluble extract is then often neutralized (or acidified) to precipitate hemicelluloses. Organic solvents are also commonly used instead of (or in addition to) acidification to precipitate further hemicellulose fractions.

Aqueous extracts of many hemicellulose fractions are known to form gels (or viscous media) when treated with oxidizing agents. phenomenon is known as "oxidative gelation" in the art, but the term is used herein in a somewhat broader sense to include the case where viscous solutions are produced rather than true gels. This reflects the fact that oxidative gelation is a progressive phenomenon which may be controlled to vary the degree of gelation to the extent that hard, brittle gels are formed at one extreme and slurries, gravies or viscous liquids at the other.

The biochemical basis of the gelling process is not yet fully understood. However, gel formation and/or viscosity increases are thought to arise (at least in part) from cross linking within and/or between macromolecular components of the hemicellulose mediated by ferulic acid residues (for example, involving diferulate generated by oxidative coupling of the aromatic nucleus of ferulic acid). These ferulic acid residues occur on arabinoxylans present in the hemicellulose. Extensive hydrolysis (by e.g.

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harsh alkaline treatments) is known to strip the ferulic acid residues from the bulk pentosans, and so hemicelluloses for use as starting materials in the production of gels or viscous solutions are usually extracted by water (particularly hot water) or mild alkali extraction.

As used herein (and as is usual in the art), the terms "ferulic acid" and "ferulate" are used sensu lato encompass feruly! (often denoted feruloy!) groups (i.e. 4-hydroxy-3-methoxy-cinnamyl groups) and derivatives (particularly oxidized derivatives) thereof.

Only a few oxidizing agents are known to have the ability to induce gelation, and these include hydrogen peroxide (usually in conjunction with a peroxidase), ammonium persulphate and formamidine disulphide.

WO 96/03440 describes the use of an oxidase (preferably a laccase) for promoting oxidative gelation of inter alia arabinoxylans. However, laccase may not be acceptable for use in certain food applications, is relatively expensive and the supply is limited. Moreover, oxidases such as laccase are relatively weak oxidation-promoters, and the range of different gel strengths obtainable by the use of such enzymes is limited. Indeed it is possible that the crosslinking achieved through the use of laccase and other oxidases differs fundamentally from that mediated by e.g. hydrogen peroxide, so that the gels may differ significantly in structure from those produced by other forms of oxidative gelation.

WO 93/10158 describes oxidative gelation of hemicellulosic material using an oxidizing system comprising a peroxide (such as hydrogen peroxide) and an oxygenase (such as a peroxidase). However, hydrogen peroxide is inconvenient as a reagent in industrial-scale processes, and is potentially dangerous.

There is therefore a need for alternative methods of promoting

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oxidative gelation which avoid the aforementioned problems.

Thus, according to the present invention there is provided a hemicellulosic material comprising an oxidase (e.g. glucose oxidase) and optionally a peroxidase (e.g. horse radish peroxidase) supplement.

The hemicellulose/hemicellulosic material for use in the invention may be any hemicellulose meeting the definition set out earlier. In particular, the hemicellulose may be an arabinoxylan, heteroxylan or pectin. In addition, the hemicellulose for use in the processes of the invention may be a synthetic hemicellulose (i.e. a structural analogue of a naturally-occurring hemicellulose synthesised in vitro by any chemical/enzymic synthesis or modification).

Thus, any non-cellulosic, non-starch plant polysaccharides may be used in the process of the invention. Thus, the processes of the invention find application in the processing inter alia of pentosans, pectins and gums.

Some hemicelluloses are suitable as substrates for oxidative gelation ("gelling hemicelluloses"); such hemicelluloses often have substituents with phenolic groups which are cross-linkable with certain oxidizing agents. These "gelling" hemicelluloses are particularly preferred for use in the invention. Non-gelling hemicelluloses may be first derivitized with phenolic (e.g. ferulic) acid groups prior to use in the invention.

Arabinoxylans, heteroxylans and pectins may also be used. Of the arabinoxylans, particularly preferred are AXFA, AXF, AXA and AX.

Also suitable for use in the invention are pectins, including the true pectins, simple pectins, complex pectins, mesocomplex pectins and gelling pectins (e.g. those obtainable from representatives of the plant family Chenopodiaceae, which include beets (e.g. sugar beet), spinach and

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mangelwurzels). Particularly preferred is sugar beet pectin (for example in the form of sugar beet pulp). Also useful in the invention are treated pectins (as hereinbefore defined).

The hemicellulosic material may be obtained by any of the standard techniques known in the art for obtaining hemicelluloses suitable as starting materials for oxidative gelation. Preferably, the hemicelluloses are obtained by any of the processes described in WO 93/10158.

As used herein, the term "supplement" as applied to any specified enzyme activity is intended to embrace not only the case where an appropriate enzyme preparation is added during production, but also encompasses the case where endogenous enzyme activity is activated, enhanced, induced or derepressed by any treatment (e.g. chemical or physical treatment) of the hemicellulosic material. Thus, the hemicellulosic material of the invention exhibit supplemental oxidase (and optionally peroxidase) activity howsoever achieved (so long as the level(s) of enzyme activity are sufficient e.g. to promote oxidative gelation), and are not essentially limited to hemicellulose preparations which have been prepared in any particular way.

Preferably, however, the enzyme supplement is added isolated enzyme having the desired activity. The level of purity and/or specificity is not crucial to the practise of the invention, so long as oxidase and/or peroxidase levels are elevated to levels sufficient to promote oxidative gelation under appropriate conditions.

As used herein, the term peroxidase denotes an enzyme which catalyses the general reaction:

 $H_2O_2 + H_2A = 2H_2O + A$ 

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where H<sub>2</sub>A is any oxidisable substrate. Without wishing to be bound by any theory, it is thought that in the case of oxidative gelation the substrate is the polysaccharide/ferulic acid complex, so resulting in crosslinking between the oxidized ferulic acid components via formation of a new C-C bond and the production of differulic acid.

Preferred according to the present invention is peroxidase EC 1.11.1.7 (e.g. horse radish peroxidase). Alternatively, naturally occurring peroxidase activity endogenous to the hemicellulose material may be exploited according to the invention.

As used herein, the term oxidase denotes an enzyme which catalyses the general reaction:

where AH2 is glucose and the enzyme glucose oxidase, the reaction is:

$$O_2 + H_2O_3 + glucose \Rightarrow H_2O_3 + gluconic acid$$

Preferred according to the present invention is glucose oxidase EC 1.1.3.4. (e.g. A. niger as source. Other oxidases which are suitable for use in the invention include amino acid oxidases, diamine oxidases and xanthine oxidase.

The gelation system of the invention avoids the dangers associated with excess of hydrogen peroxide (which carries a risk of explosion): in the gels of the invention a "negative feedback" loop ensures that if temperature rises due to excessive hydrogen peroxide production then the enzymes producing the hydrogen peroxide are progressively denatured as the temperatures rises, so limiting the production of further hydrogen peroxide.

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The gellation system of the invention also has further important and unexpected advantages which inter alia permit the formulation of self-felling powders or solutions.

The material of the invention may further comprising an exidase substrate (e.g. glucose) supplement. If glucose is used, then this has the ancillary advantage of acting as a dispersant. Alternatively, endogenous substrates naturally present in the hemicellulose may be exploited.

The hemicellulosic material may be derived from any of a wide range of different starting materials. Sultable starting materials containing hemicellulose for use in the invention typically include plant material of various kinds and any part or component thereof.

Plant materials useful as a starting material in the invention include the leaves and stalks of woody and nonwoody plants (particularly monocotyledonous plants), and grassy species of the famile Gramineae. Particularly preferred are gramineous agricultural residues, i.e. the portions of grain-bearing grassy plants which remain after harvesting the seed. Such residues include straws (e.g. wheat, oat, rice, barley, rye, buckwheat and flax straws), corn stalks, corn cobs and corn husks.

Other suitable sterting materials include grasses, such as prairie grasses, gamagrass and foxtail. Other suitable sources include dicotyledonous plants such as woody dicots (e.g. trees and shrubs) as well as leguminous plants.

Another preferred source are fruits, roots and tubers (used herein in the botanical sense). The term "fruit" includes the ripened plant ovary (or group thereof) containing the seeds, together with any adjacent parts that may be fused with it at maturity. The term "truit" also embraces simple dry fruits ifollicles, legumes, capsules, achenes, grains, samaras and nuts

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(including chestnuts, water chestnuts, horsechestnuts etc.)), simple fleshy fruits (berries, drupes, false berries and pomes), aggregate fruits and multiple fruits. The term "fruit" is also intended to embrace any residual or modified leaf and flower parts which contain or are attached to the fruit (such as a bract). Encompassed within this meaning of fruit are cereal grains and other seeds. Also contemplated for use as starting materials are fruit components, including bran, seed hulls and culms, including malt culms. "Bran" is a component of cereals and is defined as a fraction obtained during the processing of cereal grain seeds and comprises the lignocellulosic seed coat as separate from the flour or meal. Other suitable component parts suitable as starting materials include flours and meals (particularly cereal flours and meals, and including nonwoody seed hulls, such as the bracts of oats and rice).

The term "root" is intended to define the usually underground portion of a plant body that functions as an organ of absorption, aeration and/or food storage or as a means of anchorage or support. It differs from the stem in lacking nodes, buds and leaves. The term "tuber" is defined as a much enlarged portion of subterranian stem (stolon) provided with buds on the sides and tips.

Preferred lignocellulosic starting materials include waste stream components from commercial processing of crop materials such as various beets and pulps thereof (including sugar beet pulp), citrus fruit pulp, wood pulp, fruit rinds, nonwoody seed hulls and cereal bran. Suitable cereal sources include maize, barley, wheat, oats, rice, other sources include pulses (e.g. sova), legumes and fruit.

Other suitable starting materials include pollen, bark, wood shavings, aquatic plants, marine plants (including algae), exudates, cultured tissue, synthetic gums, pectins and mucilages.

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Particularly preferred as a starting material is testaceous plant material, for example waste testaceous plant material (preferably containing at least about 20% of arabinoxylan and/or glucoronoarabinoxylan).

The starting material may be treated directly in its field-harvested state or (more usually) subject to some form of pre-processing. Typical preprocessing steps include chopping, grinding, cleaning, washing, screening, sieving etc.

Preferably, the starting material is in a substantially ground form having a particle size of not more than about 100 microns. It may be air classified or sieved (for example to reduce the level of starch). Alternatively, or in addition, the starting material may be treated with enzymes to remove starch (e.g. alpha- and/or beta-amylase). The starting material may also be pre-digested with a carbohydrase enzyme to remove &-glucan.

Suitable washing treatments include washing with hot water or acid (e.g. at a pH of 3-6, e.g. about 5). This at least partially separates protein. Other pre-treatments include protease treatment.

The hemicellulosic material may, for example, be obtained from cereal husk or bran, or legumes, e.g. from maize, wheat, barley, rice, oats or malt, though any source of hemicellulose may be used in the invention so long as it is subject to at least some degree of oxidative gelation.

Preferably, the hemicellulosic material comprises a pentosan, e.g. a water soluble or alkali soluble pentosan fraction. Particularly preferred are materials wherein the pentosan comprises arabinoxylan, for example arabinoxylan ferulate. In one preferred embodiment, the hemicellulose of the invention consists (or consists essentially) of arabinoxylan ferulate.

The material of the invention preferably takes the form of a powder,

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for example a substantially anhydrous powder. Powders according to the invention preferably contain a dispersant (e.g. glucose or maltodextrin). Such powders are conveniently formulated so as to be self-gelling on the addition of water in the presence of air, for example being formulated to contain oxidase, oxidase substrate (e.g. glucose) and optionally peroxidase supplements.

The invention also contemplates the material as described herein in the form of an aqueous solution. For some applications, such aqueous solutions are preferably oxygen free and packaged in containers which effectively exclude oxygen. Such solutions may be formulated so as to be is self-gelling on exposure to oxygen (e.g. the oxygen in ambient air), for example being formulated to contain oxidase, oxidase substrate (e.g. glucose) and optionally peroxidase supplements.

Also contemplated by the invention are gals or viscous media comprising the material of the invention which has been oxidatively galled. Such gals or viscous media may comprise (or consist essentially of) cross linked arabinoxylan.

The invention also contemplates a process for preparing a gel or viscous medium comprising the step of oxidatively gelling the materials of the invention, for example by adding water to the anhydrous self-gelling powders or by exposing the oxygen free solutions to air or oxygen.

In another aspect, the invention contemplates a process for effecting oxidative gelation of a hemicellulosic material comprising the step of promoting the generation of hydrogen peroxide in situ by redox enzymes.

The redox enzymes preferably comprise an oxidase (e.g. glucose oxidase) and a peroxidase (e.g. horse radish peroxidase), which are preferably present as supplements in the hemicellulosic material.

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According in this aspect of the invention, the process may comprise the steps of supplementing a hemicellulosic material with an oxidase and optionally an exidase substrate and/or a peroxidase. The generation of hydrogen peroxide is then preferably promoted by:

- providing oxygen to the material (e.g. by generating oxygen in (a) situ); and/or
- providing water to the material; and/or (b)
- (c) providing oxidase substrate to the material (e.g. by generating substrate in situ); and/or
- activating one or more of the redox enzymes (e.g. chemically or physically), wherein the provision of oxygen or substrate may be by controlled release or generation in situ, for example triggered generation or release by heat, irradiation or chemical treatment.

Where the oxygen is provided by triggering chemical production in situ, the invention finds particular application in retort cooking when the gel can be induced to form only on heating.

The invention also contemplates a gel or viscous medium produced by (or obtainable by) any of the processes of the invention.

In another aspect, the invention contemplates a process for producing a hemicellulosic material comprising the step of supplementing a hemicellulose with an oxidase (e.g. glucose oxidase) and optionally a peroxidase, (e.g. horse radish peroxidase), and also contemplates materials produced by (or obtainable by) such a process.

The hemicellulose products (i.e. the gels, dehydrated gels, rehydrated dehydrated gels, gelling (but ungelled) hemicelluloses and viscous liquids of the invention find a variety of applications various therapeutic, surgical, prophylactic, diagnostic and cosmetic (e.g. skin care) applications.

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For example, the aforementioned materials may be formulated as a pharmaceutical or cosmetic preparation or medical device, for example selected from: a wound plug, wound dressing, wound debriding system, controlled release device, an encapsulated medicament or drug, a lotion, cream (e.g. face cream), suppository, pessary, spray, artificial skin, protective membrane, a neutraceutical, prosthetic, orthopaedic, ocular insert, injectant, lubricant or cell implant matrix. The non-gelling, gelling and gelled hemicelluloses (e.g. AX, AXF and gelled AXF) are particularly useful as agents which maintain the integrity of the gut wall lining, and as agents for coating the luminal wall of the gastrointestinal tract. They may therefore fins particular application in animal feeds and in the treatment of gastrointestinal disorders.

In such embodiments the material, gel or viscous medium of the invention may further comprising an antibiotic, electrolyte, cell, tissue, cell extract, pigment, dye, radioisotope, label, imaging agent, enzyme, co-factor, hormone, cytokine, vaccine, growth factor, protein (e.g. a therapeutic protein), allergen, hapten or antigen (for e.g. sensitivity testing), antibody, oil, analgesic and/or antiinflammatory agent (e.g. NSAID).

Thus, the above-listed materials find application in therapy, surgery, prophylaxis or diagnosis, for example in the treatment of surface (e.g. skin or membrane lesions, e.g. burns, abrasions or ulcars). In a particularly preferred embodiment, the invention contemplates a wound dressing comprising the above listed materials of the invention, for example in the form of a spray. Such wound dressings are particularly useful for the treatment of burns, where their great moisture retaining properties help to prevent the wound drying out.

Particularly preferred for such application is a self-gelling liquid comprising gelling hemicellulose supplemented with glucose and peroxidase and/or oxidase enzymes which gels on contact with oxygen in the air. Such

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compositions can be provided in the form of oxygen-free liquids in airtight containers which can be sprayed onto the skin, whereupon the liquid gels after exposure to the air. Such composition may advantageously be formulated so as to produce a slight excess of hydrogen peroxide on exposure to oxygen, so that a sterilizing, antibacterial, bacteriostatic and/or cleansing effect is obtained which helps promote healing.

The invention also contemplates water absorbent napples, diapers, incontinence pads, sanitary towels, tempons and panty liners comprising the above-listed materials, as well as domestic and industrial cleaning or liquid (e.g. water) recovery operations (e.g. in the oil industry).

Alternatively, the gels of the invention can be provided in the form of hydrated or dehydrated sheets or pellicles for application to various internal or external surfaces of the body, for example during abdominal surgery to prevent adhesions.

Other applications include enzyme immobilizing systems, brewing adjuncts and bread improvers.

The materials listed above also find application as a foodstuff, dietary fibre source, food ingredient, additive, lubricant, supplement or food dressing. Such products are preferably selected from crumb, alginate replacer, cottage cheeses, aerosol toppings, frozen yoghurt, milk shakes, ice cream, low calorie products such as dressings and jellies, batters, cake mixes, frozen chips, binders, gravies, pastas, noodles, doughs, pizza toppings, sauces, mayonnaise, jam, preserve, pickles, relish, fruit drinks, a clouding agent in drinks, syrups, toppings and confectionary (e.g. soft centres) perfood (wherein the gel e.g. acts as a bindor), a flavour delivery agent, a canning gel, fat replacer (e.g. comprising macerated gel), a coating, a glaze, a bait, a binder in meat and meat analogue products (for example vegetarian products), an edible adhesive, a gelatin replacer or dairy product

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or ingredient (e.g. a yoghurt supplement).

When used as a fat replacer the gel of the invention is preferably macerated to optimize its mouthfeel and fat mimetic properties.

The ungelled (but gellable) hemicelluloses (e.g. AXF) find particular application as clouding agents (e.g. in drinks), as film forming agents (e.g. in moisture barriers), glazes, edible adhesives and other functional food ingredients.

The invention will now be described by reference to the following examples which are purely exemplary and which do not limit the scope of the invention in any way.

## Example 1

1.0g of a maize-derived hemicellulosic powder prepared according to the processes described in WO 93/10158 was mixed with 0.5g of glucose and 20mg each of peroxidase and glucose oxidase (Sigma). composition gelled at 2% in water within 5 min on shaking in air.

## Example 2

0.3g of a composition prepared as described in Example 1 was mixed with 6g of Regent (heat-treated) wheat flour and dispersed as a batter (3g of flour mix in 9g of water). The product became a solid gel in about 10 min.

## Example 3

ig of the flour mix prepared as described in Example 2 was mixed with a further 5g of Regent flour and dispersed as a batter (3g of flour mix

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in 9g of water). The product became a solid gel in about 30 min.

# Example 4

1.0g of a maize-derived hemicellulosic powder prepared according to the processes described in WO 93/10158 was mixed with 0.25g of glucose and 10mg each of peroxidase and glucose oxidase (Sigma). The composition gelled at 2% in water in 8 min on shaking in air.

## Example 5

1.0g of a maize-derived hemicellulosic powder prepared according to the processes described in WO 93/10158 was mixed with 0.125g of glucose and 5mg each of peroxidase and glucose oxidase (Sigma). The composition gelled at 2% in water in 45 min on shaking in air.

# Example 6

1.0g of a maize-derived hemicellulosic powder prepared according to the processes described in WO 93/10158 was mixed with 0.063g of glucose and 2.5mg each of peroxidase and glucose oxidase (Sigma). The composition gelled at 2% in water after 2 hours on shaking in air.

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